DATA EVALUATION RECORD

STUDY 5

CHEM 074801 Tribufos §162-2

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 42007205
Stevenson, T.S., and W.M. Leimkuehler. 1990. The Metabolism of Tribufos in Soil Under Anaerobic Conditions. Mobay Report No. 100333. Unpublished study performed and submitted by Mobay Corporation, Stilwell, KS.

DIRECT REVIEW TIME = 12

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CONCLUSIONS:

Metabolism - Anaerobic Soil

1. This study is acceptable and fulfills the anaerobic soil metabolism data requirement.

2. Tribufos degraded slowly in sandy loam soil that was incubated in the dark at 25 ± 1°C under anaerobic conditions (nitrogen atmosphere) for 60 days following 30 days of aerobic incubation. An anaerobic half-life of 389 days was calculated. The primary degradate identified was 1-butane sulfonic acid.

METHODOLOGY:

Sandy loam soil (58% sand, 27% silt, 15% clay, 3.8% organic matter, pH 6.8, CEC 18 meq/100 g) was sieved (2 mm), dried to 75% of 0.33 bar

moisture, and subsamples (100 g dry weight) were transferred to 250-mL Erlenmeyer flasks. The soil was treated at 7 ppm with butyl-labeled [1-^{14}C]tribufos (radiochemical purity 97.9%, specific activity 20.4 mCi/mMol), dissolved in acetone. The solvent was allowed to evaporate, the soil was mixed, and the flasks were sealed with glass stoppers and parafilm. The flasks were wrapped in aluminum foil to exclude light, and the samples were aerobically incubated at 25 ± 1°C for 30 days. At 15 and 30 days posttreatment, the flasks were purged with air; the air exiting the flasks was vented through dry ice-cooled methanol, methanol, and 1 M NaOH trapping solutions, and then through activated charcoal (Figure 2). After 30 days, the flasks were purged with nitrogen to create an anaerobic environment. The flasks were also purged with nitrogen at 45, 61 and 90 days posttreatment (15, 31 and 60 days of anaerobic incubation); the nitrogen was vented through the trapping system described above. Soil moisture was adjusted to 75% of field capacity as necessary. Duplicate flasks were removed immediately posttreatment, at 30 days, 45 (15 days of anaerobicity), 61 (31 days of anaerobicity), and 90 days posttreatment (60 days of anaerobicity).

The soil samples were extracted with acetonitrile using an oscillating shaker; the slurry was separated by vacuum filtration and aliquots of the extract were analyzed by LSC. The extracted soil was then extracted twice with methanol; the slurries were separated and analyzed as described above. Each extract was then concentrated (method not reported). Aliquots of the acetonitrile and combined methanol extracts were analyzed by TLC on silica gel plates developed in hexane:acetone (9:1, v:v). Reference standards of tribufos, dibutyl disulfide, 1-butane sulfonic acid, and methyl-des butylthio tribufos were cochromatographed with the extracts and were visualized by UV (254 nm) or by treatment with sulfuric acid, heating, and charring. Radioactive areas were detected by autoradiography and/or a linear radioactivity analyzer. Methanol extracts of the 31-day anaerobic soil were also analyzed by reverse phase TLC on KC18F plates developed in acetonitrile:methanol:0.5 NaCl (40:40:20, v:v:v). Areas of interest were scraped from the plate, eluted with methanol, concentrated to dryness (method not reported), redissolved in water, and analyzed by HPLC. Aliquots of the concentrated extracts and the eluant of the individual radioactive areas from the TLC plates were analyzed by HPLC using a C-18 column and gradient mobile phase of acetonitrile/tetrahydrofuran (85:15, v:v):0.1% acetic acid. Additional aliquots were analyzed for polar compounds by HPLC using an anion exchange column (RP-18) and a gradient mobile phase of 0.5 N NaCl and water. The eluate from the columns was monitored by a radioactivity detector. Column fractions containing radioactive compounds were concentrated to dryness and the compounds were redissolved in methanol for analysis by GC/MS.

The extracted soil was then refluxed with 1 N HCl. The slurry was separated by filtration, and the refluxate was concentrated (method not reported). Aliquots of the refluxate were analyzed by HPLC using

an anion exchange column (RP-18) and a gradient mobile phase of 0.5 N NaCl and water. The eluate from the columns was monitored by a radioactivity detector. Eluate fractions containing radioactive compounds were concentrated to dryness and the compounds were redissolved in methanol for analysis by MS. Subsamples of the extracted soil were analyzed by LSC following combustion. Aliquots of the trapping solutions were analyzed by LSC; subsamples of the activated charcoal were analyzed by LSC following combustion.

DATA SUMMARY:

Butyl-labeled [1-^{14}C]tribufos (radiochemical purity 97.9%), at 7 ppm, degraded slowly in sandy loam soil incubated anaerobically (nitrogen atmosphere) in the dark at 25 ± 1°C for up to 60 days following a 30-day aerobic incubation period. Tribufos was 106.7-106.8% of the applied radioactivity immediately posttreatment, 90.7-94.5% at 30 days (day 0 of anaerobicity), and 73.0-84.4% at 90 days (60 days of anaerobicity; Appendix C). A 389 day anaerobic half-life was calculated. The degradates identified were

1-butane sulfonic acid,

which was detected at maximums of 3.4% of the applied at 61 days posttreatment (31 days of anaerobic incubation) and 3.5% at 90 days (60 days of anaerobic incubation); and,

methyl-des butylthio tribufos,

which was a maximum of 0.4% at 61 days posttreatment (31 days of anaerobic incubation). Organic volatiles comprised 0.2% of the applied radioactivity at 30 days posttreatment and 0.5% at 90 days posttreatment (60 days of anaerobic incubation); carbon dioxide was 0.4% by the end of the study. Acid reflux of the extracted soil released an additional 0.2-3.0% of the applied radioactivity which cochromatographed with methyl-des butylthio tribufos. Unextracted radioactivity increased from 0.4-0.5% of the applied immediately posttreatment to 4.4-5.3% at 30 days posttreatment, and 7.5-11.7% at 90 days.

The material balances were 91.1-107.4%.

COMMENTS:

1. The registrant-calculated half-life for tribufos of 389 days is of limited value because the calculations involve extrapolation considerably beyond the experimental time limits of the study. Data are often incapable of accurately predicting trends outside of their range because small differences are magnified and reactions which appear to be linear may, in fact, be curvilinear.

APPENDIX C

Distribution of Radioactivity in [^{14}C] Tribufos Treated Soil Maintained Under Anaerobic Conditions for 31 Days

Percent of Total Radioactivity Applied to Soil Sample[1]

Compound	Aerobic Phase				Anaerobic Phase					
	0		30[2]		15		31		60	
	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
Tribufos	106.8	106.7	94.5	90.7	92.2	89.0	87.0	88.3	73.0	84.4
1-butane sulfonic acid[3]	0.0	0.0	2.9	2.4	2.7	3.5	2.8	3.4	2.5	3.5
Des-butyl[4] Tribufos	-	-	-	-	-	-	0.4	0.4	0.0	0.0
Organic Volatiles	0.0	0.0	0.2	0.2	0.4	0.4	0.5	0.5	0.5	0.5
2	0.0	0.0	0.2	0.2	0.1	0.2	0.3	0.3	0.4	0.4
Acid[5],[6] Released	0.2	0.2	0.8	1.2	1.0	1.5	1.4	1.5	3.0	2.2
Bound	0.4	0.5	4.4	5.3	4.2	4.4	6.3	6.9	11.7	7.5
Total	107.4	106.7	103.1	100.0	100.6	99.0	98.7	101.3	91.1	98.5

[1] Percentage values are actual data.
[2] 30-day aerobic is also 0-day anaerobic data.
[3] Confirmed by solvent system.
[4] Co-chromaotgraphed with methyl-des butylthio tribufos in TLC system 3.
[5] 1 N HCl reflux for 1 hour.
[6] Acid relased ^{14}C activity is des butyl tribufos.Appendix C

TABLE I

Soil Analysis[1]

Mobay Soil Number	0383
Textural Analysis (%)	
Sand	58
Silt	27
Clay	15
Class	Sandy Loam
Organic Matter (%)[2]	3.8
pH (in 0.01 *M* $CaCl_2$	6.8
Cation Exchange Capacity[3] (meq./100 g)	18
Particle Density (gm/cc)[4]	2.6

[1]Notebook Reference: 86-R-57 p. 95

[2]% Organic matter based on total organic carbon * 1.9

[3]CEC determined by using sodium acetate, pH 8.2

[4]Particle density determined by use of Mobay Ag Chem Report No. 67681

TABLE II

Retention Characteristics for Tribufos and Its Soil Metabolites
In TLC[1] Solvent Systems

Compound	TLC Rf System 1[2]	TLC Rf System 2[2]	TLC Rf System 3[3]
Tribufos (Vial 491)	0.25	0.21	0.33
Dibutyl Disulfide (Vial 494)	0.27	0.51	0.28
1-Butane Sulfonic Acid-sodium salt (Vial 495)	-	-	0.87
Methyl-des Butylthio DEF (Vial 493)	-	-	0.70

[1]TLC solvent systems:
1. Hexane/Acetone (9:1)
2. Hexane/ Ethyl Acetate (85:15)
3. Acetonitrile/Methanol/0.5 N Sodium Chloride (40:40:20)

[2]Rf values on Whatman Analytical 0.25-mm silica plates
[3]Rf values on Whatman KC18F 0.25-mm reverse phase TLC
[4]Number in parenthese is Mobay Standard ID number.

TABLE III

HPLC Solvent Systems and Retention Times for the Analysis of Tribufos and Metabolites

Compound	Retention Time (minutes) System 1[1]	System 2[2]
Tribufos	26	--
Dibutyl Disulfide	28	--
1-Butane Sulfonic Acid	--	21
Methyl-des Butylthio Tribufos	--	5

[1]System 1; Column, Econosil C-18, 10μ, 250 mm x 4.6 mm; Solvent A, ACN/ tertrahychofuran (85:15), Solvent B, water/acetic acid (99.9:0.1); 0 time 50% B, 15 minutes, 20% B, 30 minutes 10% B, 35 minutes 5% B.

[2]System 2; Column, Alltech anion exchange mixed mode RP-18, 7μ, 250 x 4.6 mm; Solvent A, 0.5 *N* NaCL, Solvent B, water; 0 time 100% B, 10 minutes 50% B, 20 minutes 50% B, 30 minutes 0% B, 40 minutes 0% B.

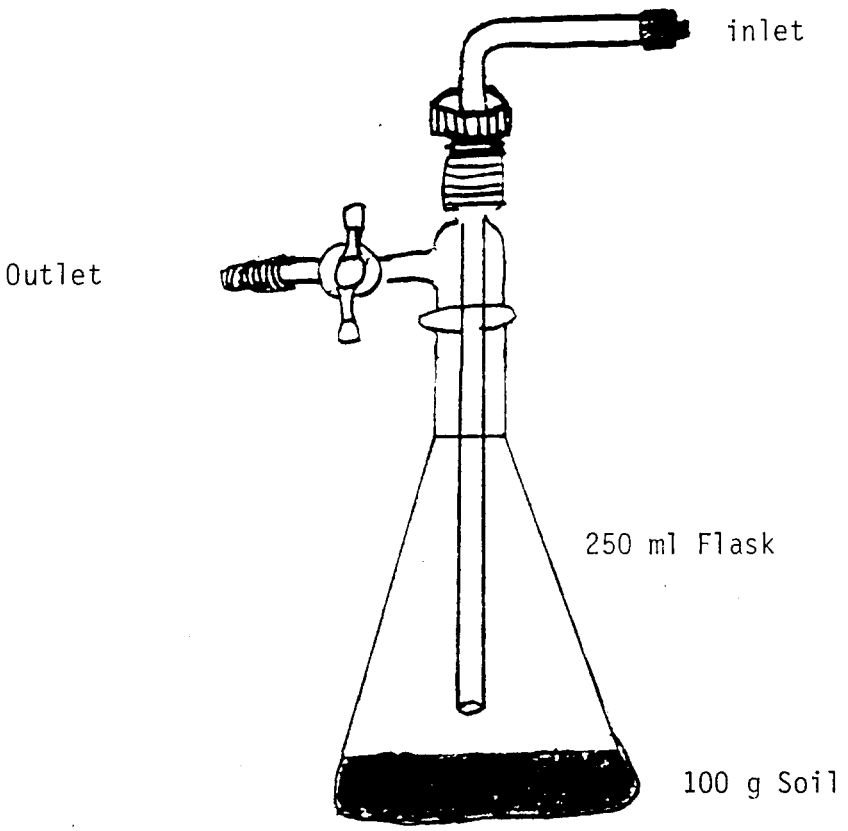

Figure 1. Diagram of flask and valve assembly used for the anaerobic soil metabolism study. During the study the flask was wrapped in aluminum foil.

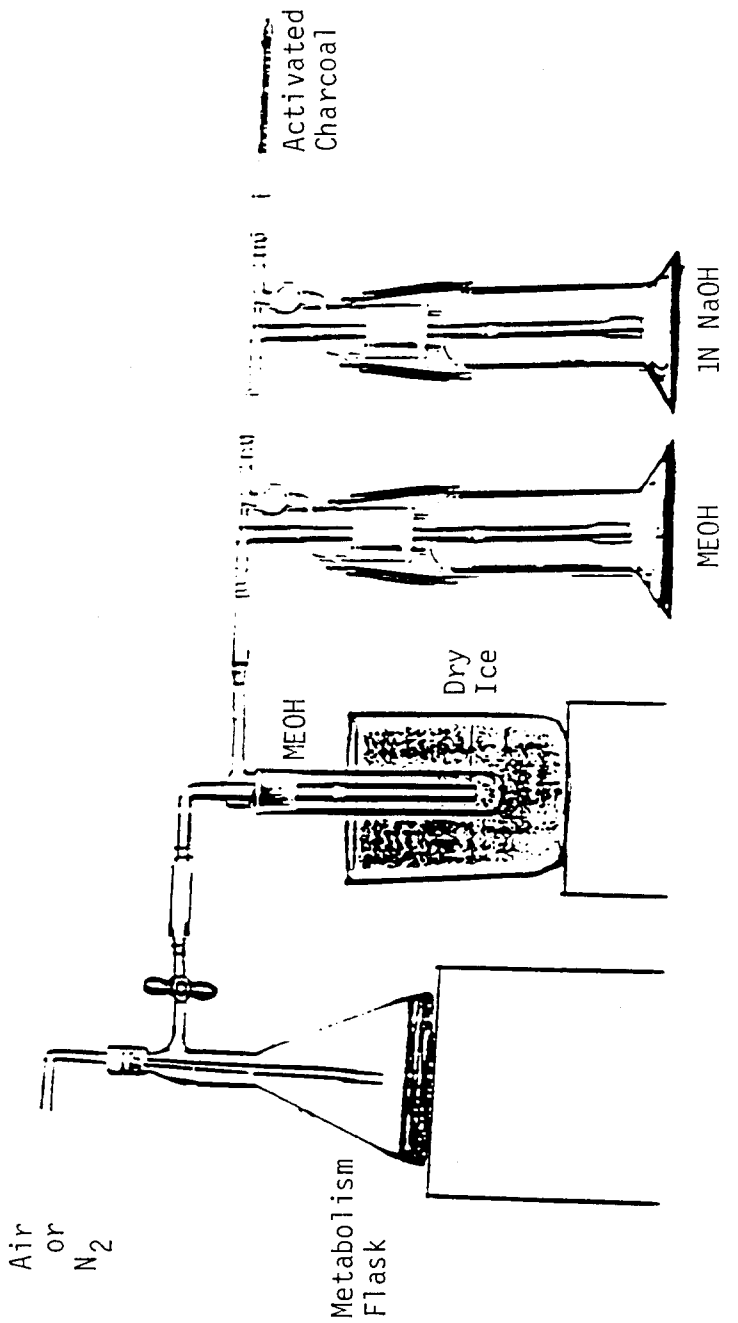

Figure 2. Trapping system used to trap volatile [^{14}C] material.

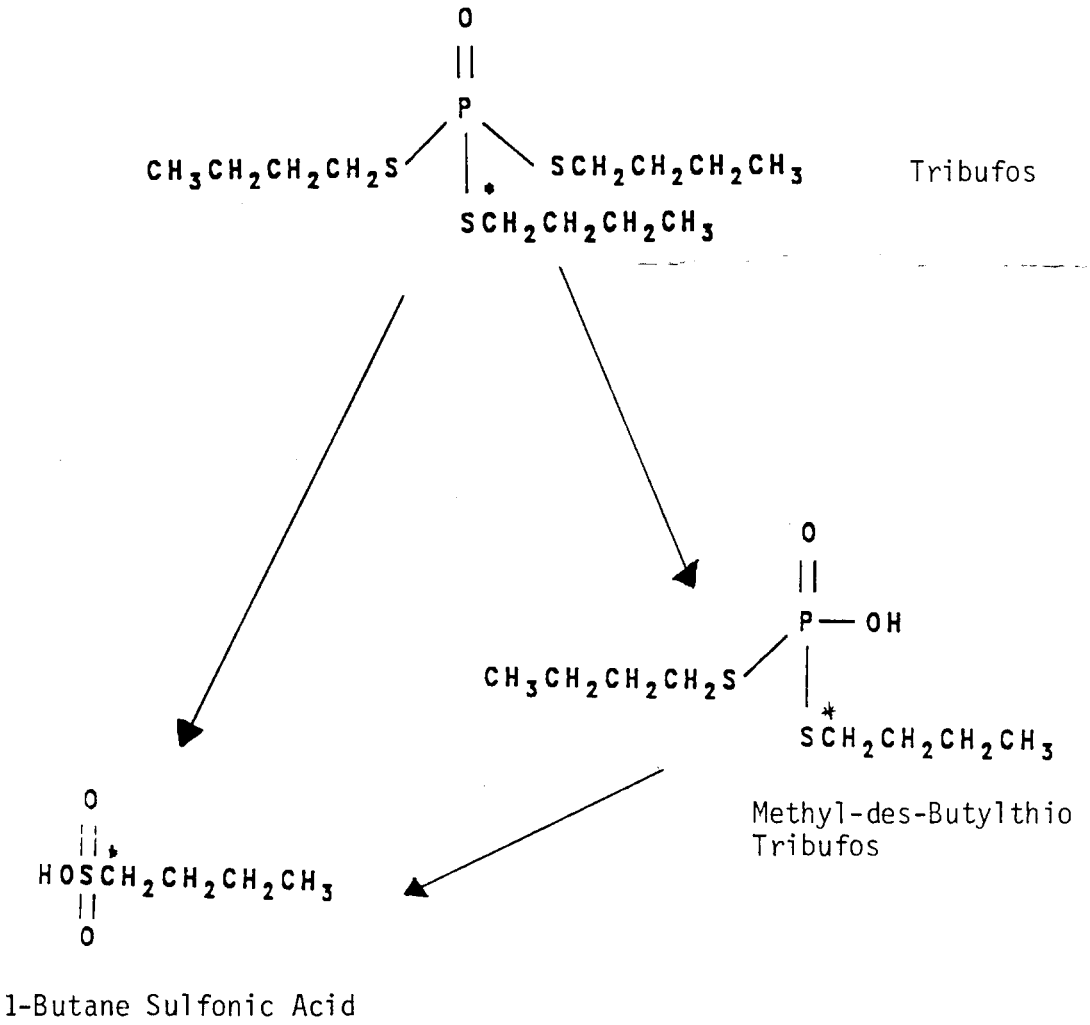

Figure 8. Proposed metabolic pathway for the degradation of tribufos in sandy loam under anaerobic conditions.